A-D

PCT/GB98/01722

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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
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ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 02 February 2000 (02.02.00)	
International application No. PCT/GB98/01722	**Applicant's or agent's file reference** HMJ03113WO
International filing date (day/month/year) 12 June 1998 (12.06.98)	**Priority date** (day/month/year)
Applicant JONES, Richard, Henry et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

27 December 1999 (27.12.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

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PCT/GB98/01722

TENT COOPERATION TRE Y

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NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

GILL JENNINGS & EVERY
Broadgate House
7 Eldon Street
London EC2M 7LH
ROYAUME-UNI

Date of mailing (day/month/year)
18 January 2000 (18.01.00)

IMPORTANT NOTIFICATION

Applicant's or agent's file reference
HMJ03113WO

International application No.
PCT/GB98/01722

International filing date (day/month/year)
12 June 1998 (12.06.98)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

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3. Further observations, if necessary:

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PCT/GB98/01722

TENT COOPERATION TRE Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

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(54) Title: INSULIN ANALOGUE

(57) Abstract

A novel analogue of insulin has covalently conjugated thereto, preferably at the B1 residue, 3,3',5'–triiodothyroxine. The conjugate is believed to be hepatoselective, whilst it retains insulin receptor binding properties.

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INSULIN ANALOGUE

The present invention relates to novel insulin analogues which are covalent conjugates of an insulin molecule and a derivative of the hormone thyroxine, 3,3',5'triiodothyronine.

In WO-A-95/05187 we described novel insulin conjugates with hormones, specifically with tetraiodothyroxine (3,3',5,5'tetraiodothyronine, T4), which were hepatoselective. The hepatoselectivity was believed to be due to the fact that, when introduced percutaneously, the size of the molecule (about 15% higher molecular weight than insulin itself) allows it to diffuse through the capillary endothelium into the circulation. In the circulation it is believed to bind reversibly the circulating proteins having an affinity for the thyroxine moiety, namely throxine binding globulin, thyroxine binding prealbumin and albumin, collectively known as thyroxine binding proteins (TBP). These higher molecular weight complexes are then unable to diffuse back through capillary endothelium, but are able to diffuse through the relatively larger pores of the hepatic endothelium. The conjugate is found to retain insulin activity. The hepatoselectivity ensures that insulin is directed to the site where its activity is required.

In WO-A-95/07931 hydrophobically modified insulin analogues are described. The insulin is generally derivatised by acylation of the pendant amino group of lysine at B29 with a fatty acid. However there is also an example of derivatising that residue with thyroxine, or with tetraiidothyroacetic acid. The analogues are alleged to have a protracted profile of action, although the mechanism by which this takes place is not elucidated.

One potential problem with the T4-insulin conjugate is that it may retain thyroxine activity. The present invention seeks to solve this problem while providing a conjugate which retains its hepatoselectivity, insulin activity and circulating protein affinity.

A new compound according to the invention comprises an insulin molecule covalently bound to 3,3',5'-triiodothyronine.

The 3,3',5'triiodothyronine molecule is not a naturally occurring compound. It is an isomer of 3,5,3' triiodothyronine (T3) and is consequently known as reverse T3, rT3. It has insignificant activity on thyroxine receptor, but thyroxine binding proteins have an affinity for the molecule. Thus the compound of the invention should have affinity for TBP's and, it is believed, consequential hepatoselectivity whilst the compound and its metabolites should not stimulate thyroxine activity.

The rT3 moiety should be conjugated to a residue of the insulin molecule such that insulin activity is not adversely affected. As in WO-A-95/05187, conjugation is preferably through the B1 residue of insulin. Alternatively the B29 residue may be linked to rT3. In WO-95/07931, the B29 residue may be derivatised and the methods of conjugating a carboxylic acid-containing compound to the B29 residue as disclosed in that reference may be used in the present invention.

The insulin may be made by recombinant DNA techniques or may be isolated from natural sources, human or animal. Recombinant insulin may have deleted residues as desired, for instance the B29 residue may be deleted. Other residues of naturally occurring insulin may be substituted, usually by conservative substitutions. For instance in WO-A-95/07931, analogues in which the B3 and/or A21 residues are other than those of naturally occurring insulin.

The rT3 molecule is conjugated to the insulin using conventional biochemical techniques in which pendant groups on the appropriate residue of the insulin molecule are covalently bonded to rT3, through the carboxylate group. The pendant group is usually the ε-amino group of a lysine residue. Any other lysine residues may be rendered unreactive by protecting the ε-amine groups using

conventional techniques. Protecting groups are removed after conjugation to the rT3 molecule.

The phenolic OH group of rT3 is protected during the process, also.

Either or both of the amine group and the carboxylate group may be activated prior to contact of the insulin with the rT3. Conventional techniques for generation of amide linkages may be used, for instance using known reagents.

A spacer may be included between the insulin molecule and the rT3 molecule. A spacer may, for instance, improve retention of insulin activity and/or TBP-binding. A spacer may also be used to control *in vivo* cleavage and metabolism of the conjugate compound, and consequently its insulin activity. A spacer may, for instance include a chain comprising 2 to 22 carbon and/or heteroatoms, such as a 4-10 atom chain, preferably comprising an alkylene group and carbonyl and/or amino groups, amido groups and or oxygen atoms in ester or ether linkages.

The inventors have found that the insulin-rT3 conjugate has a similar potency relative to human insulin itself. This is in contrast to T4-insulin, which appears to have a greater potency than human insulin. In the presence of binding proteins, especially thyroxin binding proteins, the potency of T4-insulin is reduced, whereas these proteins do not affect the potency of rT3-insulin. These data indicate that the conjugate is likely to have similar effects as insulin *in vivo*.

Further tests in which the ED50 of the conjugates as compared to insulin, in the presence and absence of binding proteins (human serum albumin and thyroxin binding globulin and transthyretin) show that each conjugate on its own has a similar ED50 to human insulin itself. The ED50's of the T4-insulin conjugate are significantly increased by the presence of TBG, whilst the ED50's of the rT3-insulin are not effected to a significant degree.

We have also conducted competitive binding assays of the insulin analogues compared to human insulin with

^{125}-Insulin to insulin receptors on liver plasma membrane (LPM). Insulin is known to inhibit the binding of ^{125}-Insulin to these receptors. We have found that TBP does not affect this ability. rT3 behaves in a similar way to human insulin in that it inhibits binding of ^{125}-Insulin to the receptors on LPM and this is not affected by the presence of TBP. T4 insulin itself does inhibit ^{125}-Insulin binding to these receptors. In contrast, however, TBP significantly affects this inhibition.

The novel compound is suitable for use in a method of treatment of the human or animal, for instance to replace insulin in a method of insulin replacement therapy. The invention thus comprehends novel compositions containing the compound as well as pharmaceutical compositions containing the compound and a pharmaceutically acceptable excipient. The composition is formulated so as to be suitable for administration by the usual routes, generally by subcutaneous injection. Accordingly the carrier is generally aqueous. The invention comprehends also a new use of the compound in the manufacture of a medicament for use in a method of treatment of the human or animal body.

The following examples illustrate the invention.

Example 1
Preparation of [rT3(Na-B1)]-insulin

1.1 Synthesis of Msc-rT3

50.0 mg rT3 (76.8 umol, 651.0 g/mol)
20.4 mg Msc-OSu (76.9 umol, 265.24 g/mol)

50.0 mg rT3 were suspended in 400 ul dimethylformamide and 20.4 mg Msc-OSu, dissolved in 100 ul dimethylformamide, were added. 4 ul of triethylamine were pipetted into the solution and the mixture was stirred overnight at room temperature.

1.2 Synthesis of Msc-rT3-OSu

16.6 mg DCC (80.6 umol, 206.3 g/mol)

16.6 md DCC were dissolved in 50 ul dimethylformamide and added to the above reaction mixture. The activation is complete after 3 h at room temperature.

1.3 Synthesis of [rT3(Na-B1)]-insulin

230 mg A1,B29-(Msc)2-insulin (6078 g/mol, 38 umol) synthesised according to Schüttler A and Brandenburg D, Hoppe-Seyler's Z. Physiol.Chem. 360, 1721-1725 (1979) were dissolved in 3 ml dimethylformamide with the addition of 4 ul triethylamine and then reacted with 69 ug Msc-rT3-OSu (898 g/mol, 76 umol, two-fold excess with respect to insulin derivative). After stirring for 3 h at room temperature the acylation was stopped by addition of 50 ul acetic acid. The solution was dialysed overnight against distilled water and lyophilised. For cleavage of Msc protecting groups the protein material was diluted in a mixture of 1 ml dimethylformamide, 1.5 ml methanol and 1.5 ml water. The solution was cooled to 0°C and addition of 0.5 ml of ice-cold 2 M sodium hydroxide solution started the cleavage reaction. The reaction was stopped by acidification with 1 ml of 10% (v/v) acetic acid. The protein was precipitated by pipetting the reaction solution into a mixture of 250 ml of ice-cold ether and 20 ml methanol and stirring for 1 h. The ether was decantated from the precipitated protein and the protein dried in vacuo.

Purification of the raw material was performed by use of RP-MPLC. Fractions were collected and lyophilised.

Chromatographic conditions:

Column: RP20C18, 2.5 x 250 mm, 122 ml total volume,

Gradient: 25-40% (v/v)

2-propanol in water containing 0.1% trifluoro acetic acid, total gradient volume 1.5 l; flow rate 20 ml / 3 min.

Yield: 27 mg (10% of theory, based on A1,B29-(Msc)2-insulin)

Molecular mass: 6437 u (calc. 6436.6 u)

Purity (RP-HPLC): 93 % (Absorption at 215 nm)

1.4 Mass spectrometry

MS-TOF spectrometer VG TofSpec, Fisons

Ionisation: Ar-laser, MCP Volts,: 1750, 337 nm, linear modus Acceleration: 20 kV

Standard: bovine insulin 5731 u (calc. 5731 u), vasointestinal peptide 1424 u (calc. 1426 u) [rT3(Na-B1)]-insulin: 6437 (calc. 6437)

Example 2 - Effects of Binding Proteins on Receptor Binding

The rT3-insulin conjugate made in Example 1 is used in various tests to determine the binding potencies of the analogues on liver plasma membrane. ^{125}-Insulin is used as the labelled insulin. It is known that insulin itself inhibits binding of ^{125}-Insulin.

Results

Equilibrium binding curves

The equilibrium binding curves of average normalised bound against the log-concentration of insulin or analogue (nmol/l) with or without the presence of THBP were generated. The trends initially illustrated by the curves were:

H-Ins, rT3-Ins and T4-Ins appear similar in their positions, i.e. there is no difference between them in their ability to inhibit the binding of ^{125}-Insulin to insulin receptors on LPM.

The presence of THBP does not appear to affect the ability of H-Ins to inhibit the binding of ^{125}-Insulin to insulin receptors on LPM.

The presence of THBP does not appear to affect the ability of rT3-Ins to inhibit the binding of ^{125}-Insulin to insulin receptors on LPM.

The presence of THBP does appear to affect the ability of T4-Ins to inhibit the binding of ^{125}-Insulin to insulin receptors on LPM as shown by the shift in the T4-Ins+THBP curves to the right. TBG seems to have the greatest effect on T4-Ins, i.e. causes the greatest shift.

ED50

The ED50's as calculated by the G-PIP software were inverse logged because the concentrations entered in G-PIP had to be entered as the log of the concentrations. The average (nmol/1)± SEM of the ED50's was then calculated. The results are shown in Table 1. These give a quantitative idea of the shift, if any in the equilibrium binding curves.

TABLE 1

Average of ED50 ± SEM			
	Average	**SEM**	**n=**
H-Ins	1.966	0.43	5
rT3-Ins	2.455	0.35	6
0.5% HSA	2.48	0.478	4
1% HSA	3.24	0.379	3
2.5% HSA	2.76		2
Transthyretin	1.805	0.55	4
0.135μmol/l TBG	3.147	0.35	3
T4-Ins	1.316	.034	5
0.5% HSA*	3.715		2
1% HSA*	5.823	2.108	3
2.5% HSA*	4.81		2
Transthyretin*	2.935	0.32	4
0.135μmol/l TBG*	21.67	2.258	3
0.27μmol/l TBG*	36.55		2

* Fisher's test also performed.

Statistical analysis of the ED50's

From the statistical analysis it was found that the ED50's of rT3-Ins and T4-Ins were not significantly different from that of H-Ins. The ED50's of rT3-Ins with THBP were not significantly different from those of rT3-Ins without THBP present as determined by ANOVA. On the other hand, the ED50's of T4-Ins without THBP present ($p<0.05$) as determined by Fisher's least squares test (see Table 1*).

Potency estimates

The potency estimates of the analogues relative to H-Ins and the analogues in the presence of THBP relative to the analogues in the absence of THBP are shown in Table 2 with their fiducial limits. This demonstrates that rT3-Ins has a similar potency relative to H-Ins. T4-Ins seems to have a greater potency relative to H-Ins. The presence of THBP seems to have no effect on the binding potency estimates of rT3-Ins binding to insulin receptors relative to rT3-Ins without THBP present. However the presence of THBP present. However the presence of THBP greatly reduces the T4-Ins binding potency estimates relative to T4-Ins binding to insulin receptors without THBP present (Table 2).

TABLE 2

Potency Estimates		
	Potency	95% fiducial limits
H-Ins	100%	
rT3-Ins	94%	56-157
T4-Ins	184%	111-318
rT3-Ins	100%	
0.5% HSA	122%	87-173
1% HSA	87%	58-129
2.5% HSA	119%	80-178
0.135μmol/l TBG	76%	54-107
Transthyretin	183%	111-306
T4-Ins	100%	
0.5% HSA	27%	15-46
1% HSA	31%	16-54
2.5% HSA	35%	19-60
0.135μmol/l TBG	5%	2-9
Transthyretin	33%	20-54

Scatchard Plots

The Scatchard plot of H-Ins demonstrates the characteristic curvilinear shape of negative co-operativity that should be exhibited by human insulin. It may be seen from the Scatchard plots of rT3-Ins and T4-Ins that these analogues also exhibit negative co-operativity due to their curvilinear shape.

Reference Example - Synthesis of Insulin - T4

The T4 insulin is B1-thyroxyl-insulin made according to the technique described in WO-A-95/05187, Example 1.

CLAIMS

1. A compound consisting of an insulin molecule covalently bound to 3,3',5' triiodothyromine.

2. A compound according to claim 1 in which the 3,3',5' triiodothromine is bound to a lysine residue of the insulin molecule.

3. A compound according to claim 2 in which the 3,3',5' triiodothyromine is bound to the B1 lysine residue.

4. A compound according to any preceding claim in which the insulin is human insulin.

5. A compound according to any preceding claim for use in a method of treatment of the human or animal body.

6. A composition comprising a compound according to any of claims 1 to 4 and a carrier.

7. A pharmaceutical composition comprising a compound according to any of claims 1 to 4 and a pharmaceutically acceptable excipient.

8. Use of a compound according to any of claims 1 to 4 in the manufacture of a composition for use in a method of treatment of the human or animal body.

9. Use according to claim 8 in which the method is insulin replacement therapy, preferably for treatment of diabetes.

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/GB 98/01722

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/62 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and. where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 05187 A (UNITED MEDICAL & DENTAL SCHOOL ;DEUTSCHES WOLLFORSCHINST (DE)) 23 February 1995 cited in the application see abstract ---	1-8
A	WO 95 07931 A (NOVO NORDISK) 23 March 1995 cited in the application see abstract see examples -----	1-8

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Panzica, G

1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9505187 A	23-02-1995	EP 0725648 A	14-08-1996
		JP 10501789 T	17-02-1998
		US 5854208 A	29-12-1998
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		PL 313444 A	08-07-1996
		SK 32496 A	06-11-1996
		US 5750497 A	12-05-1998
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PATENT COOPERATION TREATY

From the
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Applicant's or agent's file reference HMJ03113WO	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/01722	12/06/1998	12/06/1998

Applicant
KINGS COLLEGE LONDON

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Vullo, C Tel. +49 89 2399-8061

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
HMJ03113WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/01722	12/06/1998	12/06/1998
International Patent Classification (IPC) or national classification and IPC		
C07K14/62		
Applicant		
KINGS COLLEGE LONDON		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27/12/1999	28.06.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Halle, F Telephone No. +49 89 2399 8537

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/01722

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-9 as originally filed

Claims, No.:

1-9 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-9
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-9
	No:	Claims	

2. Citations and explanations

see separate sheet

Section V

Having regard to the prior art, the subject-matter of claim 1-9 appears to be novel (Article 33(2) PCT). The prior art documents WO-A-95 05187 and WO-A-95 07931 (both cited in the application), which are considered to represent the most relevant state of the art, disclose insulin analogues which are covalent conjugates of an insulin molecule and a covalently bound thyroxine-derived molecule. A technical problem by using thyroxine-derived molecules is that the insulin conjugate may retain thyroxine activity. This problem appears to be solved by the present invention by using a covalent insulin conjugate wherein the thyroxine-derived molecule is 3,3',5'-triiodothyronine; the cited prior art does not refer to such an insulin conjugate.

The subject-matter of claims 1-9 also appears to involve an inventive step (Article 33(3) PCT). Since the problem with the remaining thyroxin activity has been solved, the proposed invention is not only an alternative covalent insulin conjugate but also an advantageous conjugate solving the problem of thyroxine activity while retaining in particular its insulin activity. Since no indication has been made in the prior art to obtain such an advantageous insulin conjugate, the claimed invention is not obvious to a person skilled in the art.

PATENT COOPERATION TREA

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
HMJ03113WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 98/ 01722	12/06/1998	
Applicant		
KINGS COLLEGE LONDON		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 2 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application.
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title**,
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/01722

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/62 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 05187 A (UNITED MEDICAL & DENTAL SCHOOL ;DEUTSCHES WOLLFORSCHINST (DE)) 23 February 1995 cited in the application see abstract ---	1-8
A	WO 95 07931 A (NOVO NORDISK) 23 March 1995 cited in the application see abstract see examples -----	1-8

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 February 1999	10/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Panzica, G

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

[redacted]rmation on patent family members

[redacted]ternational Application No
PCT/GB 98/01722

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9505187 A	23-02-1995	EP 0725648 A	14-08-1996
		JP 10501789 T	17-02-1998
		US 5854208 A	29-12-1998
WO 9507931 A	23-03-1995	AU 4846197 A	19-02-1998
		AU 682061 B	18-09-1997
		AU 7652094 A	03-04-1995
		BG 61611 B	30-01-1998
		BG 100420 A	31-12-1996
		BR 9407508 A	07-01-1997
		CA 2171424 A	23-03-1995
		CN 1133598 A	16-10-1996
		CZ 9600789 A	16-10-1996
		EP 0792290 A	03-09-1997
		FI 961220 A	14-05-1996
		HU 75991 A	28-05-1997
		JP 9502867 T	25-03-1997
		NO 961070 A	15-05-1996
		NZ 273285 A	24-10-1997
		PL 313444 A	08-07-1996
		SK 32496 A	06-11-1996
		US 5750497 A	12-05-1998
		ZA 9407187 A	17-03-1995